An animal model of fibrous dysplasia

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Fibrous dysplasia of bone (FD) is the most severe clinical feature of the McCune–Albright syndrome (MAS) and leads to crippling skeletal disease. Activating missense mutations of the *GNAS1* gene, which encodes the α subunit of the stimulatory G (Gs) protein, underlie MAS-associated FD, and most likely many non-MAS associated, monostotic or polyostotic FD. These mutations occur post-zygotically, leading to a somatic mosaic state. Owing to these mutations, both the differentiation of bone marrow stromal cells and the function of mature bone-forming osteoblasts are severely altered, leading to the replacement of normal bone/marrow with abnormal bone and abnormal marrow, which together comprise an individual FD lesion. Matrix composition and structure are characteristically altered, and the abnormal marrow is characterized by depleted hematopoiesis, loss of marrow adipocytes, and the accumulation of fibroblast-like cells that display a pre-osteoblastic phenotype, commonly referred to as fibrous tissue (Fig.1d)[1].

Progenitor cells of skeletal tissues (osteogenic 'stem' cells) are among the adherent cell population that is isolated *in vitro* from post-natal bone marrow (reviewed in Ref. 2). These cells (colony forming unit-fibroblastic, CFU-F) are clonogenic and can differentiate into multiple phenotypes (cartilage, fat, bone, hematopoiesis-supportive stroma and fibrous tissue). When transplanted into immunocompromised mice using appropriate carriers, stromal cells can generate an ectopic 'ossicle' – a miniature bone/marrow organ in which bone and the hematopoietic microenvironment are reproduced with the appropriate spatial architecture (Fig. 1a)[3].

Thus we hypothesized that, following isolation in culture and transplantation *in vivo*, mutated bone marrow stromal cells from FD might lead to the development of an abnormal FD ossicle (Table 1). To test this hypothesis, current procedures for isolating normal human CFU-F were applied to the abnormal fibrotic marrow of FD lesions. CFU-F were efficiently released from the abnormal marrow of the FD lesions using this approach. Taking advantage of the clonogenic property of CFU-F, mutation analysis using a variety of techniques could be extended to the level of individual colonies formed by CFU-F obtained from FD tissues. The consistent detection of mutated and non-mutated colonies in cultures of FD-derived CFU-F demonstrated that individual fibrous dysplastic lesions are non-clonal lesions in which mutated bone marrow stromal cells and their progeny coexist with their normal counterparts. Therefore, an individual FD lesion can be regarded as a somatic mosaic itself. Both multi-colony-derived strains of stromal cells that reflect a mosaic of normal and mutated bone

Table 1. Features of fibrous dysplastic (FD) bone compared with an ectopic ossicle generated by transplantation of human bone marrow stromal cells derived from FD lesions into immunocompromised mice

FD bone	Animal model of human FD	Comments
Reduced amounts of bone	Reduced amounts of bone	Bone marrow stromal cells differentiate into bone-forming osteoblasts but are unable to form normal quantities of bone.
Qualitative abnormalities of bone (e.g. woven bone and Sharpey fibers)	Qualitative abnormalities of bone (e.g. woven bone and Sharpey fibers)	In normal post-natal bone, new bone is deposited primarily colinearly with the bone-forming surface to generate lamellar bone. In FD, primarily woven bone is deposited and collagen fibers are perpendicular to the bone-forming surface, forming Sharpey fiber bone.
Lack of hematopoiesis	Lack of homing of hematopoietic cells	Hematopoiesis (which is of recipient origin) cannot be established because the transplanted cells fail to establish a hematopoiesis-supportive stroma.
Loss of adipocytes	Lack of adipogenesis	In development of a bone/marrow organ, adipogenesis occurs after hematopoiesis is established. Since hematopoiesis is blocked, adipogenesis is also blocked.
Fibrosis in intra-trabecular spaces	Fibrosis between bone and ceramic particles	Because of inefficient bone formation, bone marrow stromal cells accumulate, thereby replacing what would normally become marrow.
Lesions comprise normal and mutated cells (somatic mosaics)	Lesions are generated by transplantation of normal and mutated cells	Lesions are 'somatic mosaics', comprising normal and mutated cells rather than a clonal expansion of mutated cells. In the transplants, FD ossicles form only when a mixture of normal and mutated cells are used. Clones of mutant cells fail to generate a lesional ossicle.

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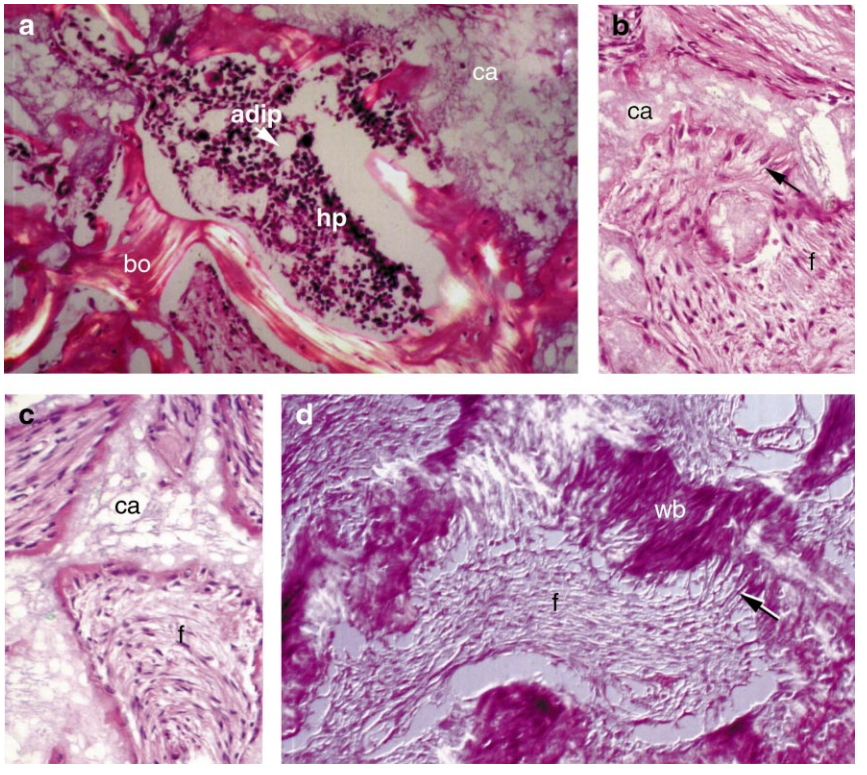

Figure 1. Histological features of ossicles, generated by subcutaneous transplantation of normal and fibrous dysplastic bone marrow stromal cells into immunocompromised mice, and of fibrous dysplastic bone. Bone marrow stromal cells from bone and marrow of normal age-matched donors were expanded *in vitro*, attached to ceramic particles containing hydroxyapatite/tricalcium phosphate and transplanted subcutaneously into immunocompromised mice. After six weeks, a complete bone/marrow organ has been regenerated. (**a**) Bone (bo) was deposited on the surfaces of carrier particles (ca), and a fully functional marrow complete with hematopoiesis (hp) and adipocytes (adip) developed. (**b**,**c**) In contrast, bone marrow stromal cells derived from patients with fibrous dysplasia of bone (FD) did not form a normal ossicle. The fibrous dysplastic cells deposited only limited amounts of bone, resulting in the deposition of collagen fibers perpendicular to, rather than colinear with, the surface and in the formation of Sharpey fiber bone (arrows). Spaces between the particles were filled with a fibrotic tissue (f), which did not support the establishment of hematopoiesis or adipogenesis. These features are virtually identical to (**d**) the histological features of FD tissue, which contains abnormal woven bone (wb) and Sharpey fiber bone (arrows) deposited by misfunctioning osteoblasts. Furthermore, the marrow spaces are completely obliterated by an over abundance of fibrous tissue (f).

marrow stromal cells, and clonal strains derived from mutated colonies, were thus generated and used for *in vivo* transplantation experiments.

Control strains of normal stromal cells, when transplanted into immunocompromised mice using hydroxyapatite-based carriers, generated a normal ossicle in which substantial amounts of primarily lamellar bone were deposited onto carrier surfaces. Normal hematopoiesis of host origin was established in the ossicle, and the full range of cell types comprising the hematopoietic microenvironment were consistently observed (Fig. 1a)[3]. In contrast, transplantation of mosaic strains of stromal cells derived from MAS patients generated an abnormal ossicle in which hematopoiesis was never established and marrow adipocytes never developed (Fig. 1b,1c). The amount of bone formed in these transplants was significantly lower compared to controls, and displayed structural abnormalities characteristic of FD, including the predominance of woven bone and the characteristic prominence of 'Sharpey fibers.' The human origin of the tissues that formed was proven by *in situ* hybridization with human-specific *alu* sequences. In essence, all the cardinal histopathological features of FD (Fig. 1d) were reproduced in the MAS transplants. Unlike multi-colony-derived strains, clonal strains of mutated skeletal stem cells did not generate either a normal or an FD ossicle, but were lost from the transplant as indicated by the absence of human (*alu*-positive) cells[4].

We learned from these experiments that mutated bone marrow stromal cells can effectively be used to generate miniature versions of FD, thus providing a unique animal model of the disease based on the *in vivo* differentiation and function of abnormal human cells. The same approach might be easily applied to other genetic diseases of the skeleton and might provide insight into the mechanisms that link genotype to phenotype as a result of abnormal osteogenic cell differentiation and function. We also learned that a mosaic of normal and mutated cells is not only found in the individual human FD lesion *in situ* but is also required to generate an FD miniature in our assay. Besides providing experimental proof of Happle's hypothesis of somatic mosaicism as a means for survival of the otherwise lethal *GNAS1* mutation[5], these data indicate that somatic mosaicism is a necessary condition for the development of FD and point to important interactions between normal and mutated osteogenic cells within an individual lesion.

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